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Subinhibitory concentrations of antibiotics affect biofilm formation in *Listeria monocytogenes*

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Background

The Gram-positive bacteria *Listeria monocytogenes* has a pathogenic and saprophytic life-style and can be exposed to subinhibitory antibiotic concentrations in both niches. These concentrations affect gene expression and physiology such as biofilm formation, cell morphology etc. in several pathogenes[1]. We have previously found that subinhibitory antibiotic concentrations alter stress and virulence gene expression and cause enhanced stress sensitivity but do not affect Caco-2 cell invasion[2]. The effect of conventional antibiotics on biofilm formation is unknown although several studies have investigated effect of novel antimicrobials.

Purpose

The aim of this study was to determine whether subinhibitory antibiotic concentrations affect *L. monocytogenes* biofilm formation and if so, determine potential mechanisms.

Methods and Materials

Biofilm formation was investigated in BHI broth at 30 and 37°C and quantified using crytal violet (O'Toole & Kolter assay). Bacterial counts were determined of the planktonic part of the culture.

Swimming motility was investigated when exposed to 2-fold concentration of antibiotics in BHI with 0.25% agar at 25 and 37°C.

Biofilm formation

Subinhibitory concentration of ampicillin and gentamicin significantly reduced the biofilm formation at 37°C (Fig. 1A) whereas the same antibiotic increased the biofilm formation at 30°C (Fig. 1B). At both 30 and 37°C, co-trimoxazole enhanced biofilm formation even though the bacterial count was reduced.

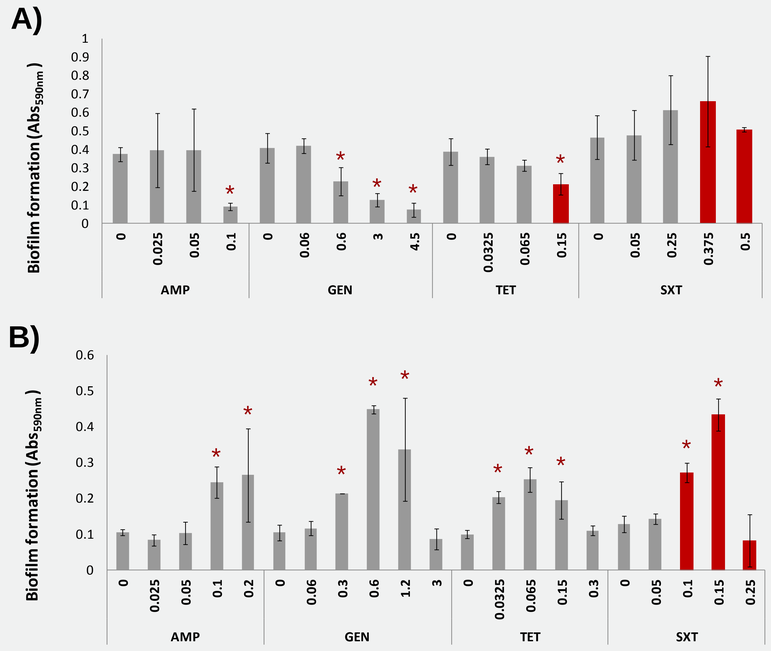

Fig.1. Subinhibitory antibiotic concentration change the biofilm formation at 37°C (A) and 30°C (B). Asterisk denote $P<0.05$ when comparing biofilm of the control to the antibiotic exposed biofilm. Red bar indicate that bacterial count was significantly reduced as compared to control.

Morphology

Subinhibitory concentrations of ampicillin and co-trimoxazole, but not gentamicin and tetracycline, caused minor changes to morphology of the planktonic cells.

Motility

Compared to the control, reduced swimming diameter was observed for all four antibiotics (Fig. 2) however, the bacterial count per plate was also reduced. Thus, the effect was caused by growth reduction of *L. monocytogenes* and not reduced motility.

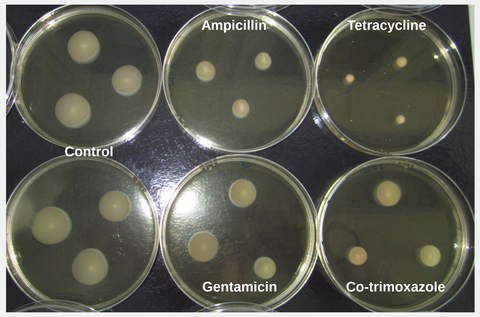

Fig.2. Swimming at 25°C in BHI with 0.25% agar with 0.125 µg/ml ampicillin, 0.125 µg/ml tetracycline, 8 µg/ml gentamicin and 0.25 µg/ml co-trimoxzole.

Conclusion and Perspective

Subinhibitory concentration of four **antibiotics affect** *L. monocytogenes* **biofilm** formation and the antibiotic effect was **temperature-dependent**. Subinhibitory antibiotic concentrations did not change **motility** and caused only minor modification of the *L. monocytogenes* **morphology** indicating that the altered biofilm capacity was caused by other mechanisms such as c-di-GMP inactivation[3], eDNA[4] or AI-2/LuxS system[5].

The **temperatur-dependent effect** of the antibiotics on biofilm formation could be advantageous for the organism as enhanced biofilm formation at temperatures below body temperature might **facilitate persistence** in the environment.

Acknowlegdement
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